Z. Säugetierkunde **63** (1998) 285–296

ZEITSCHRIFT FÜR SÄUGETIERKUNDE
INTERNATIONAL JOURNAL OF MAMMALIAN BIOLOGY

Geographic structure, gene flow, and maintenance of melanism in *Ctenomys rionegrensis* (Rodentia: Octodontidae)

By G. D'Elía, E. P. Lessa, and J. A. Cook

Laboratorio de Evolución, Facultad de Ciencias, Universidad de la República, Montevideo, Uruguay and University of Alaska Museum, Fairbanks, Alaska, USA

Receipt of Ms. 09. 07. 1997
Acceptance of Ms. 21. 01. 1998

Abstract

Ctenomys rionegrensis has three coat color morphs (melanic, agouti, and dark-backed) within its total distribution of 50×60 km area of Uruguay. The presence of two populations fixed for the melanic form is remarkable because this coat color contrasts markedly with the surrounding substrate. Starch gel electrophoresis was used to analyze variation in 20 allozyme loci assayed in 100 individuals from seven populations of *C. rionegrensis* to test the hypothesis that melanism was fixed by genetic drift in small, isolated populations. Seven loci were monomorphic (95% criterion) and no alleles correlated exclusively with a particular coat color. Average heterozygosity was H = 0.038 (range 0.022–0.058). Using pairwise comparisons of all populations, the mean number of migrants ($\hat{M}$) was 6.342 for all pairs except those involving the population at Los Arrayanes (agouti), for which the average value was 1.532. Our results indicate that gene flow in *C. rionegrensis* is sufficiently high to prevent fixation of alternative alleles exclusively by drift. The absence of a pattern of genetic variation due to isolation by distance suggests that the current distribution resulted from a recent range expansion.

Key words: Tuco-tucos, geographical structure, gene flow, genetic drift, melanism

Introduction

There are at least three possibilities that explain patterns of differentiation when populations differ in gene frequencies: 1) populations have been molded by natural selection, with the selective agents spatially structured; 2) populations show the effects of genetic drift, and 3) patterns may result from intermixing of different stocks (i. e., gene flow). These possibilities are not mutually exclusive and may operate in combination to produce the observed patterns of geographic variation.

There is full consensus concerning the feasibility of natural selection to produce geographic variation (Mayr 1963). The effects of genetic drift in randomly changing allele frequencies are also clear. Drift may fix or eliminate alleles from populations and in the process lead to divergence among populations (e. g., Gallardo and Köhler 1994). Finally, gene flow has long been considered as a homogenizing evolutionary force, although gene flow could promote divergence by dispersing new genes and gene combinations throughout a species' range. The role that gene flow plays depends both on the importance of other evolutionary forces and on the geographic distribution of the species (Slatkin 1987).

Ctenomys rionegrensis Langguth and Abella, 1970 is one of the three Uruguayan species of the diverse genus *Ctenomys* (tuco-tucos). The species occurs in a restricted area of

about 3 000 km^2 in southwest Uruguay in the Department of Río Negro. Despite its restricted distribution, three color morphs are known: melanic, agouti, and dark-backed. Melanic individuals of this species are entirely and uniformly black, whereas agouti specimens may exhibit slightly dark dorsal areas on the head (Langguth and Abella 1970 a). Dark-backed individuals are characterized by a wide mid-dorsal band of dark grey-brown hair, which runs from the snout to the base of the tail (Altuna et al. 1985). Some populations are monomorphic for the melanic morph, others for the agouti morph, while others are comprised exclusively of dark-backed individuals. Populations that are polymorphic with respect to pelage are known. In these, melanic and agouti or agouti and dark-backed individuals coexist.

The chromatic differentiation of *C. rionegrensis* is interesting because it occurs in a restricted geographic area. This differentiation is not correlated with different soil colors, as was suggested for *C. torquatus* by Freitas and Lessa (1984) and demonstrated for populations of pocket gophers (Geomyidae) (Patton and Smith 1990), nor with variation in other environmental characteristics. All *C. rionegrensis* populations examined live in sandy meadows, which are uniformly light colored with no obvious variation in soil or vegetation.

The genetic structure of fossorial rodents, such as *Ctenomys*, has been thought to reflect their low vagility, and therefore, low levels of gene flow. Further, the supposed small size of demes has been thought to make them more susceptible to the random effects of genetic drift in fixing or eliminating alleles (Reig 1970; Reig et al. 1990; White 1978). Since selection-based explanations of color variation have not been supported for some *C. rionegrensis* populations, fixation of melanism may be due to genetic drift (Altuna et al. 1985; Langguth and Abella 1970 b). This hypothesis predicts 1) reduction in genetic variation within melanic populations and 2) low levels of gene flow among the populations, such that genetic drift would cause divergence among populations. The first prediction would pertain only if the populations fixed for the melanic forms passed through a bottleneck (Templeton 1981; see also Barton and Charlesworth 1984). The second prediction is more general and indicates that genetic drift can be an effective agent of local differentiation (Slatkin 1987; Wright 1931; cfr. Wright 1969).

In an attempt to distinguish these forces, extensive field work has been aimed at describing the distribution of *C. rionegrensis* and the spatial frequency of pelage variants. We have begun to characterize genetic variation across populations in an effort to discriminate among the various hypotheses concerning the origin and present distribution of the melanic form. Specifically, we 1) define levels of intrapopulation genetic variation with nuclear markers (allozymes); 2) establish the levels and patterns of intraspecific microgeographic differentiation; and 3) estimate gene flow between the populations.

Material and methods

One hundred specimens of *Ctenomys rionegrensis* from seven localities were collected in 1993 and 1994 and examined (Fig. 1). Specimens from Las Cañas and Nuevo Berlín were exclusively melanic, whereas all those from Mafalda and Tres Bocas were dark-backed. Polymorphic populations included El Abrojal (4 melanic, 16 agouti) and La Guarida (8 dark-backed, 4 agouti). All specimens from Los Arrayanes were agouti; however, 3 out of 18 specimens collected in Los Arrayanes in 1983 were melanic. Those individuals were not included in this study because tissue samples were not preserved.

Individuals were captured with Gopher Ready Set traps and prepared as voucher museum specimens now archived in the collection of the Laboratorio de Evolución, Facultad de Ciencias, Montevideo. Tissues (heart, liver, kidney) were extracted immediately after sacrificing the animal and stored at –80 °C. Protein electrophoresis was performed on liver homogenates with horizontal starch gels. Electromorphs were stained using standard techniques (Tab. 1) (Selander et al. 1971; Harris and Hopkinson 1976).

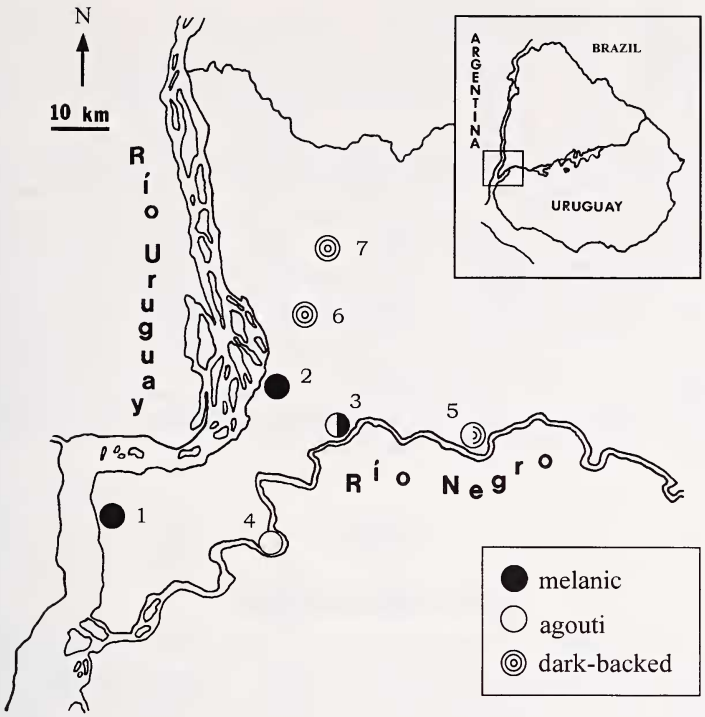

Fig. 1. Map of the studied zone. In brackets the sample sizes of the 7 populations of *Ctenomys rionegrensis*. 1) Las Cañas (16); 2) Nuevo Berlín (10); 3) El Abrojal (20); 4) Los Arrayanes (18); 5) La Guarida (12); 6) Mafalda (15); and 7) Tres bocas (9).

Table 1. Proteins, loci abbreviations, Enzyme Commission number, and electrophoretic conditions used in the study of populational differentiation in *Ctenomys rionegresis*. A = acid citrate buffer, pH 6.1/6.0, 76 mA/5 h; B = tris-citrate buffer, pH 8.0/8.0, 115 mA/5 h.

Protein	Locus abbreviation	Enzyme Commission number	Electrophoretic conditions
Adenylate kinase	ADK	(2.7.4.3)	A
Alcohol dehydrogenase	ADH	(1.1.1.1)	B
Aspartate aminotransferase	AAT1, AAT2	(2.6.1.1)	A
Glutamate dehydrogenase	GDH	(1.4.1.3)	B
Glycerol 3-phosphate dehydrogenase	α-GPD	(1.1.1.8)	B
Isocitrate dehydrogenase	ICD1, ICD2	(1.1.1.42)	A
Lactate dehydrogenase	LDH1, LDH2	(1.1.1.27)	B
Malate dehydrogenase	MDH1, MDH2	(1.1.1.37)	A
Malic enzyme	ME	(1.1.1.40)	B
Phosphoglucomutase	PGM1	(2.7.5.1)	B
Phosphogluconate dehydrogenase	6-PGD	(1.1.1.44)	A
Superoxide dismutase	SOD1, SOD2	(1.15.1.1)	A
General protein	GP1, GP2, GP3		A

Differences in the mobility of electromorphs were assumed to have a genetic basis and to follow rules of simple Mendelian inheritance. Side by side comparisons were carried out to confirm the identity of electromorphs across gels. Alphabetic designations were assigned to the different electromorphs of each presumptive locus, with a representing the fastest migrating electromorph in both anodal and cathodal systems.

BIOSYS-1 (Swofford and Selander 1981) was used to assess the average number of alleles per locus, (A), the percentage of polymorphic loci, (P), average heterozygosity per individual per observed population, (H), Wright's F-statistics and Nei's (1978) and Rogers' (1972) genetic distances. Option 5 of GENEPOP 1.2 (Raymond and Rousset 1995) was used to test for statistically significant differentiation in allele frequencies between pairs of populations. The matrix of Rogers' unbiased distances was used to construct a Wagner tree in BIOSYS-1 (Swofford and Selander 1981), and to carry out a multidimensional scaling with the program STATISTICA (1995). Gene flow was estimated by two methods. First, the effective number of migrants Nm was estimated using the private alleles method (Slatkin 1985) and option 4 of the program GENEPOP 1.2 (Raymond and Rousset 1995). Second, a method based on Fst estimated the levels of gene flow between pairs of populations, with the program DIST (Slatkin 1993) using the formula $\hat{M} = [(1/Fst)-1]/4$ where θ of Weir and Cockerham (1984) was used as an estimator of Fst. Estimates of gene flow, $\hat{M}$, for each pairwise comparison were then plotted against geographic distances between populations following Slatkin (1993).

Results

Variation within populations

Genetic variation was assayed at 20 presumptive loci, which code for 12 enzymes and 3 general proteins (Tab. 2). Under the criterion that the most common allele had a frequency not higher than 95%, seven loci were monomorphic whereas this value was four under the criterion of 99%. All other loci had two or three alleles that varied in frequency among populations (Tab. 2). No alleles were exclusive to a pelage morph but some were unique to populations. ICD-2b, ME a, SOD-1a, and GP-3c were exclusive to the Los Arrayanes population, but the latter three were at frequencies of less than 0.05. GP-3a and SOD-2b were only found in La Guarida, but the latter was at less than 0.05. The proportion of polymorphic loci under the 95% criterion varied from 0.1 in Las Cañas to 0.4 in Nuevo Berlin, and the average value was 0.25.

Table 2. Allelic frequencies, mean number of alleles per locus (A), percentage of loci polymorphic (P), mean heterozygosity (H), and sample sizes for populations of *Ctenomys rionegrensis*. Loci abbreviations are defined in table 1.

Population		1 Las Cañas	2 Nuevo Berlín	3 El Abrojal	4 Los Arrayanes	5 La Guarida	6 Mafalda	7 Tres Bocas
	n	16	10	20	18	12	15	9
Locus	Allele							
ADH	a	0.688	0.500	0.600	0.861	0.667	0.833	0.833
	b	0.312	0.500	0.400	0.139	0.333	0.167	0.167
ADK	a		0.050		0.028	0.042		
	b	1.000	0.950	1.000	0.972	0.958	1.000	1.000
AAT-1	a	1.000	1.000	1.000	1.000	1.000	1.000	1.000
AAT-2	a						0.167	0.111
	b	0.969	1.000	0.950	0.750	1.000	0.833	0.889
	c	0.031		0.050	0.250			

Table 2. (Continued)

Population		1 Las Cañas	2 Nuevo Berlín	3 El Abrojal	4 Los Arrayanes	5 La Guarida	6 Mafalda	7 Tres Bocas
	n	16	10	20	18	12	15	9
GDH	a						0.033	
	b	0.906	0.900	0.900	0.917	0.833	0.934	1.000
	c	0.094	0.100	0.100	0.830	0.167	0.033	
GPD	a	0.031		0.025	0.028	0.083		
	b	0.969	1.000	0.975	0.972	0.917	1.000	0.944
	c							0.056
ICD-1	a			0.050			0.067	
	b	1.000	1.000	0.950	0.972	1.000	0.933	1.000
	c				0.028			
ICD-2	a	1.000	1.000	1.000	0.556	1.000	1.000	1.000
	b				0.444			
LDH-1	a	0.031						
	b	0.969	0.950	0.925	1.000	0.917	0.967	1.000
	c		0.050	0.075		0.083	0.033	
LDH-2	a	1.000	1.000	1.000	1.000	1.000	1.000	1.000
MDH-1	a				0.028			
	b	1.000	0.950	1.000	0.972	1.000	1.000	1.000
	c		0.050					
MDH-2	a	1.000	1.000	1.000	1.000	1.000	1.000	1.000
ME	a				0.028			
	b	1.000	1.000	1.000	0.972	1.000	1.000	1.000
PGM	a					0.083		
	b	1.000	0.950	1.000	1.000	0.917	1.000	1.000
	c		0.050					
6-PGD	a			0.025	0.028	0.042	0.033	
	b	1.000	0.950	0.975	0.944	0.958	0.967	1.000
	c		0.050		0.028			
SOD-1	a				0.028			
	b	1.000	1.000	1.000	0.972	1.000	1.000	1.000
SOD-2	a	1.000	1.000	1.000	1.000	0.958	1.000	1.000
	b					0.042		
GP-1	a	1.000	1.000	1.000	1.000	1.000	1.000	1.000
GP-2	a		0.050		0.028			
	b	0.969	0.950	0.925	0.972	0.917	1.000	1.000
	c	0.031		0.075		0.083		
GP-3	a					0.083		
	b	1.000	1.000	1.000	0.972	0.917	1.000	1.000
	c				0.028			
A		1.30	1.40	1.40	1.70	1.50	1.35	1.15
P (95%)		10.00	40.00	30.00	25.00	35.00	20.00	15.00
H		0.028	0.050	0.040	0.058	0.038	0.033	0.022
(± 1 SE)		(0.013)	(0.015)	(0.016)	(0.017)	(0.014)	(0.018)	(0.013)

The average heterozygosity per individual per population (direct count) ranged from 0.022 in Tres Bocas to 0.058 in Los Arrayanes, and the average was 0.038. These values are similar to those observed in other underground rodents: 0.038 (Nevo et al. 1990), in particular those found in *Geomys bursarius*: 0.039 (Bohlin and Zimmermann 1982), *Ctenomys maulinus:* 0.040 (Gallardo and Köhler 1992), *C. lewisi*: 0.032 (Cook and Yates 1994), and *C. australis*: 0.030 (Apfelbaum et al. 1991). In contrast, the values of *C. rionegrensis* are higher than those of *C. argentinus*: 0.000 (Sage et al. 1986); *C. frater*: 0.012, *C. steinbachi*: 0.009 (Cook and Yates 1994), and lower than those in the *C. mendocinus*: 0.065 group (Sage et al. 1986), *C. porteousi*: 0.081 (Apfelbaum et al. 1991), *C. flamarioni*: 0.175, *C. torquatus*: 0.110, *C. minutus*: 0.114, and *C.* sp: 0.141 (Moreira et al. 1991) and *Thomomys bottae*: 0.088 (Patton and Smith 1990). The value of these comparisons is relative, because the enzymatic systems used in this study are different from cited cases, except Cook and Yates (1994). This difference can bias observed heterozygosities (see Gillespie and Kojima 1968). For example, there are clear cases in which including or excluding esterase loci from the analysis drastically affects the outcome (Apfelbaum et al. 1991; Ortells and Barrantes 1994).

Wright's (1969) Fis statistics show that five loci (ADH, GDH, GPD, PGM, and GP-2) have significant positive departures from Hardy-Weinberg equilibrium in an overall analysis across all populations. Similarly, Fis values were significantly greater than zero in two populations (Los Arrayanes and La Guarida) in analyses across all loci. In all these cases, Fis values were positive, indicating a deficit of heterozygotes (Tab. 3).

Table 3. Statistically significant ($P < .05$) Fis values. Globally, two populations (Los Arrayanes and La Guarida) have a significant deviation from the Hardy-Weinberg equilibrium across all loci ($P = .0079$ and $P = .0014$).

Locus	Los Arrayanes	La Guarida	Mafalda	Across populations
ADH	.779	.651	.774	.556
GDH		1.000		.493
GPD		1.000		.337
PGM		1.000		.596
GP-2		1.000	1.000	.465
ICD-1	.575			
GOT-2				

Table 4. Estimates of the genetic distances between 7 *Ctenomys rionegrensis* populations based on allelic frequencies of 20 loci. The lower triangular matrix is Rogers' (1972) distances; while the upper triangular matrix is Nei's (1978) distances.

Population	1	2	3	4	5	6	7
1 Las Cañas		0.000	0.000	0.013	0.000	0.001	0.001
2 Nuevo Berlín	0.027		0.000	0.019	0.000	0.006	0.005
3 El Abrojal	0.015	0.025		0.015	0.000	0.003	0.003
4 Los Arrayanes	0.056	0.071	0.064		0.015	0.011	0.011
5 La Guarida	0.030	0.036	0.029	0.077		0.003	0.002
6 Mafalda	0.027	0.044	0.030	0.052	0.050		0.000
7 Tres Bocas	0.022	0.045	0.035	0.053	0.049	0.015	

Geographic variation

In some comparisons, genetic distance between pairs of populations with the same pelage color is higher than between populations with different pelages (Tab. 4). Three main groups are distinguished (Fig. 2): the Los Arrayanes population, the populations Mafalda and Tres Bocas, and the populations of Las Cañas, El Abrojal, Nuevo Berlin, and La Guarida. Los Arrayanes is the most divergent population. Pairwise comparisons of populations (Option 5 of GENEPOP, Raymond and Rousset 1995) show no significant differences in allele frequencies between populations within each group, but significant differences in comparisons of populations from different groups (results not shown). Multidimensional scaling of genetic distances (Lessa 1990) reveals neither a clinal pattern, nor any other type of simple correlation between the geographic and genetic distances (Fig. 3).

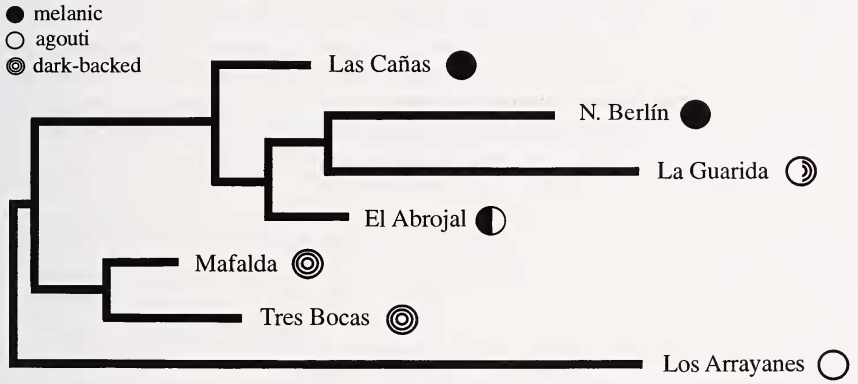

Fig. 2. Wagner tree of genetic relationships among seven populations of *Ctenomys rionegrensis* based on Roger's genetic distances (Tab. 4). The tree is rooted at midpoint of greatest patristic distance.

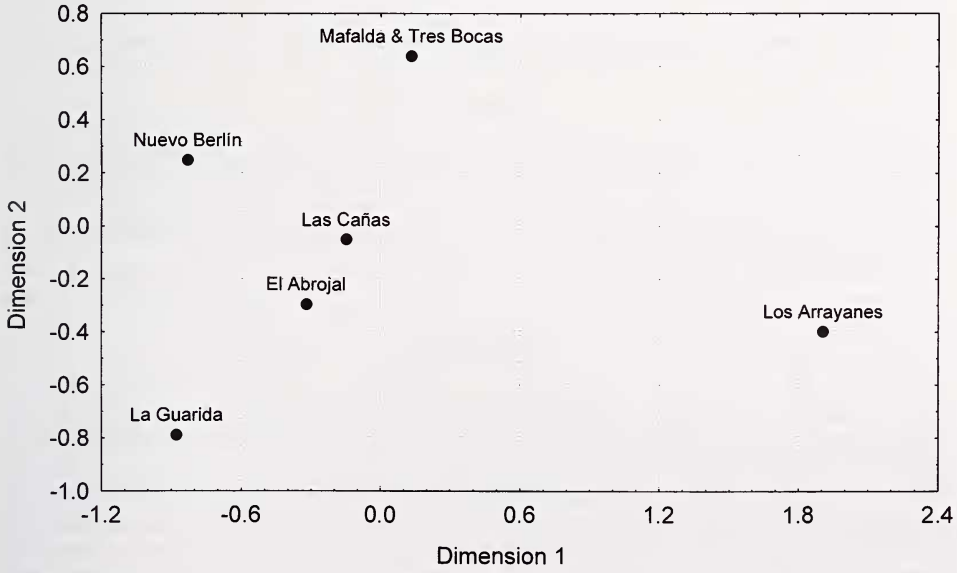

Fig. 3. Multidimensional scaling of genetic distances in two dimensions for the 7 *C. rionegrensis* populations, based on Rogers' genetic distances (Tab. 4).

The average value of Fst for all loci is 0.091, indicating that only 9.1% of the total variation is due to population subdivision. This reveals geographic subdivision on the same order as that found in *C. australis*: 0.128 (Apfelbaum et al. 1991), though lower than that estimated for *C. maulinus*: 0.330 (Gallardo and Köhler 1992), and *Thomomys bottae*: 0.258 (Patton and Smith 1990).

Gene flow

The private alleles method estimated the number of migrants per generation considering all populations to be 1.713. Slatkin's (1993) method generally showed no pattern of isolation by distance between the different populations (Fig. 4). Specifically, values of gene flow were high between the two melanic populations and between them and all other populations except Los Arrayanes. Values were lowest between Los Arrayanes and all others (average value of $\hat{M} = 1.532$). The average value of gene flow between all populations excluding Los Arrayanes was $\hat{M} = 6.342$. Figure 4 shows the relationship between geographic distance and gene flow for all pairs of populations yielding positive estimates of $\hat{M}$. Negative estimates cannot be log-transformed and are therefore missing (Fig. 4). Those values indicate extremely low genetic differentiation (Slatkin 1993).

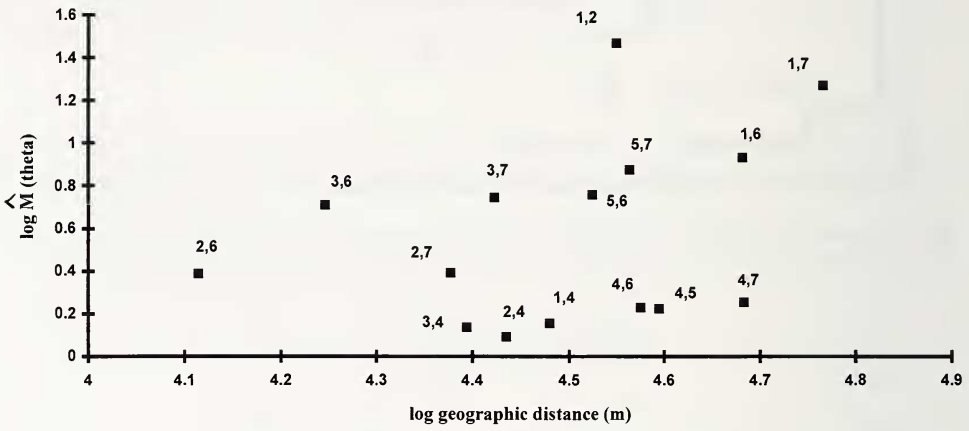

Fig. 4. Levels of gene flow ($\hat{M}$) between pairs of populations plotted against the geographic distances between them, in kilometers. The values of gene flow were estimated according to Slatkin (1993) with the formula $\hat{M} = [(1/Fst)-1]/4$, using θ of Weir and Cockerham (1984) as an estimator of Fst. The estimations of $\hat{M}$ for 6 pairs of populations do not appear because the corresponding Fst values are zero or not significantly different from zero (see text).

Discussion

Drift is considered to have a fundamental role in genetic and karyotypic evolution of subterranean rodents (Patton and Yang 1977; Sage et al. 1986). Presumed low vagility and the discontinuity of the subterranean ecotype are thought to have fragmented populations into isolated demes. Thus, a low level of gene flow exists between populations and the effects of drift and differentiation could be pronounced. With this classical image of the evolutionary dynamics of the subterranean rodents, it was thought that the fixation of melanism in some populations of *C. rionegrensis* occurred by genetic drift (Altuna et al. 1985; Langguth and Abella 1970b). This hypothesis, in turn, may be

specified in two ways: 1) invoking a bottleneck in the history of an ancestral population in which melanism became fixed; and 2) proposing generally low levels of gene flow among populations.

Our estimates of heterozygosity and polymorphism are similar in melanic and non-melanic populations. More generally, they are comparable to those found in other subterranean rodents (Nevo et al. 1990). These data suggest no reason for invoking a bottleneck that would have facilitated the fixation of melanism. The expectation in that scenario would be one of reduced genetic variability, as observed for example in Chilean populations of tuco-tucos that have experienced such events (Gallardo and Köhler 1994; but see Barton and Charlesworth 1984).

Our estimated levels of gene flow between the melanic and other populations, and generally within the species, are rather high, with the exception of Los Arrayanes, which maintains significantly lower levels of exchange and is the most genetically dissimilar population. High levels of gene flow were also found among populations of *C. australis* separated by distances between 58 and 112 km and this allowed only mild local differentiation (average value of Fst = 0.128 Apfelbaum et al. 1991).

The values of gene flow between the populations are expressed as the number of migrants per generation, Nm and pairwise values are estimated by $\hat{M}$. An average of at least one individual per generation exchanged between two populations will prevent alternative neutral alleles at the same locus from being fixed by genetic drift (Wright 1931; cfr, Wright 1969). This is independent of population size. The measures of genetic exchange in this study are indirect estimates of historial, rather than current levels of gene flow among populations (Slatkin 1987). They measure levels from some point in the species' evolutionary past, which coupled with other evolutionary forces, have shaped current patterns of genetic variation.

It seems unlikely that current levels of gene flow are as high as suggested by the observed values and here we note that the barriers currently restricting gene flow could not have existed in the past. This apparent contradiction between direct and indirect methods of estimating gene flow probably means that the species has undergone large-scale demographic changes in the recent past (Slatkin 1987). The absence of isolation by distance and in *C. rionegrensis* coupled with high values of $\hat{M}$ may indicate that these populations colonized their present ranges recently (Slatkin 1993). These populations are beginning to differentiate under the effects of isolation, but insufficient time has elapsed to permit accumulation of substantial genetic differences. The case for a recent expansion is reinforced by the low levels of population subdivision observed. Recent colonization of new areas, as postulated here, was also assumed for different karyomorphs of the "Corrientes group" of *Ctenomys*, to explain high estimates of gene flow ($\hat{M}$) (Ortells and Barrantes 1994).

Taken as a whole, our results suggest that the hypothesis of random fixation of melanism (i. e., due to drift) is unlikely. Our results are compatible with the more complex evolutionary dynamics of demographic instability. A history of recent expansion could have maintained the populations of *C. rionegrensis* far from an equilibrium of isolation by distance, in which the furthest populations would be the most differentiated ones, and in which small isolated demes are susceptible to differentiation via drift of the founder effect type of Mayr (Beatty 1992). Cycles of expansion and contraction may have been caused partially by Pleistocene marine transgressions, which are known to have reached the zone of the present distribution of *C. rionegrensis* (Alonso 1978; Sprechmann 1978). Flooding is locally known to affect some populations. In this context, the melanic populations may not be demographically independent of other populations. Gene flow may be a significant evolutionary process if movements of individuals or entire populations spread genes and combinations of genes throughout the range of a species. This situation may have produced the current geographic distribution of characters in *C. rionegrensis*. With

regards to gene flow in subterranean rodents, Patton and collaborators (Patton and Feder 1981; Patton and Smith 1990), have shown that it can vary dramatically in different circumstances. For instance, high levels of "connectedness" by gene flow are quite likely in densely populated areas, as illustrated by *C. rionegrensis*. High levels of gene flow do not lower the importance of drift; however, the fixation of melanism by drift alone is unlikely. Natural (including sexual) selection may affect pelage color, but we are far from understanding in what manner. A similar case of chromatic polymorphism, maintained by natural selection in spite of high levels of gene flow, has recently been reported in garter snake populations (Lawson and King 1996).

Combining direct sequencing and restriction enzyme analysis, we have generated preliminary data on levels of mitochondrial DNA variation in *C. rionegrensis*. Those data involved 140 individuals from nine populations including those analyzed here, Eight substitutions, defining three haplotypes, were detected. One of the haplotypes is widely distributed, while the other two have more restricted distributions. Mitochondrial DNA diversity is very low and not strongly structured, which reinforces the idea that differentiation is probably recent in populations of *C. rionegrensis*.

Finally, it is important to point out that the field work has provided valuable information about the demography of the species. For instance, chromatic polymorphisms are frequent and sometimes transient. For example, in Los Arrayanes, there was a ratio of agouti to melanic individuals in 1983 of 5 to 1. Specimens collected ten years later were exclusively agouti in spite of intensive sampling. The polymorphic populations may have originated through persistence of an ancestral polymorphism or through recent gene flow between previously isolated populations. These alternatives would indicate, respectively, that these are zones of primary or secondary contact. Preliminary estimations (made by multiplying the number of individuals captured within a quadrat by the total area occupied by the population) suggest that the populations consist of several thousand individuals. Also, we have recently found polymorphic populations with different combinations of pelage colors (e. g., melanic and agouti at La Tabaré, and all three morphs in sympatry at Portones de Chaparei). All these observations suggest that populations are more dynamic than previously thought and gene flow has had an important role in population structure. Future studies with more variable molecular markers will allow us to test hypotheses about the fixation of melanism by natural selection, and to generate a more complete picture of the evolutionary dynamics of this species.

Acknowledgements

We are grateful to Clemente "Tito" Olivera, María Noel Cortinas, Federico Hoffmann, Mariana Cosse, Ana Luz Porzecanski, Carolina and Pablo Lessa, and Nella Sanchez-Cook for their invaluable assistance in the field and to Alejandra Chiesa and Leo Joseph for reviewing earlier versions of the manuscript. Logistic support in the field was provided by the Intendencia de Río Negro and Compañía Forestal Oriental. Financial support was generously provided by CONICYT, CSIC-Universidad de la República, PEDECIBA, and the US/Uruguay Fulbright Commission.

Zusammenfassung

Geographische Struktur, Gen-Fluß und Erhaltung von Melanismus bei Ctenomys rionegrensis (Rodentia: Octodontidae)

In seinem gesamten uruguayischen 60×50 km großen Verbreitungsgebiet weist *Ctenomys rionegrensis* drei Fellfärburgen auf: melanistisch, agouti und „dorsal" dunkel. Die Anwesenheit von zwei Populationen für die melanistische Form ist erwähnenswert, da diese Färbung stark mit der Umgebung im Kon-

trast steht. Um die Hypothese zu testen, daß Melanismus in kleinen Populationen mit geringem oder keinem Gen-Fluß von benachbarten Populationen durch genetische Drift festgelegt ist, wurde Stärke-Gel Elektrophorese angewandt. Die Variation in 20 allozymen Loci von 100 Individuen aus 7 Populationen wurde geprüft. Sieben Loci waren monomorphisch (95%) und keines der Allele korreliert allein mit einer bestimmten Fellfärburg. Die durchschnittliche Heterozygotie war $H = 0.038$ (Variabilität 0.022–0.058). Der paarweise Vergleich aller Populationen ergab einen $\hat{M}$ Durchschnittswert von 6.342 für alle Paare, mit Ausnahme der Population von Los Arrayanes (agouti), dessen Durchschnittswert 1.532 erreichte. Unsere Ergebnisse zeigen, daß der Gen-Fluß bei *C. rionegrensis* ausreichend hoch ist, um der Fixierung von alternativen Allelen ausschließlich vorzubeugen. Der Mangel an einem von der Distanz abhängigen genetischen Variationmusters deutet an, daß die heutige Verbreitung das Ergebnis einer jüngeren Raumerweiterung sein könnte.

References

Alonso, C. (1978): La fauna de moluscos del yacimientos de Playa Pascual con referencia a otros yacimientos estuaricos y marinos del cuaternario de Uruguay. Com. Soc. Malacol. Uruguay **4**, 365–383.

Altuna, C. A.; Ubilla, M.; Lessa, E. P. (1985): Estado actual del conocimiento de *Ctenomys rionegrensis* Langguth y Abella, 1970 (Rodentia, Octodontidae). Actas Jor. Zool. Uruguay **1**, 8–9.

Apfelbaum, L. I.; Massarini, A. I.; Daleffe, L. E.; Reig, O. A. (1991): Genetic variability in the subterranean rodents *Ctenomys australis* and *Ctenomys porteousi* (Rodentia: Octodontidae). Biochem. Syst. Ecol. **19**, 467–476.

Barton, N. H.; Charlesworth, B. (1984): Genetic revolutions, founder effects, and speciation. Ann. Rev. Ecol. Syst. **15**, 133–164.

Beatty, J. (1992): Random drift. In: Keywords in evolutionary biology. Ed. by E. F. Keller and E. A. Lloyd. Cambridge: Harvard Univ. Press. Pp. 273–281.

Bohlin, R. G.; Zimmerman, E. G. (1982): Genic differentiation of two chromosome races of the *Geomys bursarius* complex. J. Mammalogy **63**, 218–228.

Cook, J. A.; Yates, T. L. (1994): Systematic relationships of the Bolivian tuco-tucos, genus *Ctenomys* (Rodentia: Ctenomyidae). J. Mammalogy **75**, 583–599.

Freitas, T. R. O.; Lessa, E. P. (1984): Cytogenetics and morphology of *Ctenomys torquatus* (Rodentia: Octodontidae). J. Mammalogy **65**, 637–642.

Gallardo, M. H.; Köhler, N. (1992): Genetic divergence in *Ctenomys* (Rodentia, Ctenomyidae) from the Andes of Chile. J. Mammalogy **73**, 99–105.

Gallardo, M. H.; Köhler, N. (1994): Demographic changes and genetic losses in populations of a subterranean rodent (*Ctenomys maulinus brunneus*) affected by a natural catastrophe. Z. Säugetierkunde **59**, 358–365.

Gillespie, J. H.; Kojima, K. (1968): The degree of polymorphism in enzymes involved in energy production compared to that in nonspecific enzymes in two *Drosophila ananassae* populations. Proc. Nat. Acad. Sci. USA **61**, 582.

Harris, H.; Hopkinson, D. A. (1976): Handbook of enzyme electrophoresis in human genetics. Amsterdam: North-Holland Publ. Comp.

Langguth, A.; Abella, A. (1970 a): Las especies uruguayas del género *Ctenomys*. Com. Zool. Mus. Hist. Nat. Montevideo **10**, 1–27.

– – (1970 b): Sobre una población de tuco-tucos melánicos (Rodentia-Octodontidae). Acta Zool. Lilloana **28**, 101–108.

Lawson, R.; King, R. B. (1996): Gene flow and melanism in Lake Erie garter snake populations. Biol. J. Linn. Soc. **59**, 1–19.

Lessa, E. P. (1990): Multidimensional analysis of geographic genetic stucture. Syst. Zool. **39**, 242–252.

Mayr, E. (1963): Populations, species, and evolution. Cambridge: Harvard Univ. Press.

Moreira, D. M.; Franco, M. H. L. P.; Freitas, T. R. O.; Weimer, T. A. (1991): Biochemical polymorphisms and phenetic relationships in rodents of the genus *Ctenomys* from Southern Brazil. Biochem. Genet. **29**, 601–615.

Nei, M. (1978): Estimation of average heterozygosity and genetic distance from a small number of individuals. Genetics **89**, 583–590.

Nevo, E.; Filippucci, M. G.; Beiles, A. (1990): Genetic diversity and its ecological correlates in nature: comparisons between subterranean, fossorial, and aboveground small mammals. In: Evolution of

subterranean mammals at the organismal and molecular levels. Ed. by E. Nevo and O. A. Reig. New York: Wiley-Liss. Pp. 347–366.

Ortells, M. O.; Barrantes, G. E. (1994): A study of genetic distances and variability in several species of the genus *Ctenomys* (Rodentia: Octodontidae) with special reference to a probable causal role of chromosomes in speciation. Biol. Linn. Soc. **53**, 189–208.

Patton, J. L.; Feder, J. H. (1981): Microspatial genetic heterogeneity in pocket gophers: non-random breeding and drift. Evolution **43**, 12–30.

Patton, J. L.; Yang, S. Y. (1977): Genetic variation in *Thomomys bottae* pocket gophers: macrogeographic patterns. Evolution **31**, 697–720.

Patton, J. L.; Smith, M. F. (1990): The evolutionary dynamics of the pocket gopher *Thomomys bottae*, with emphasis on California populations. Univ. California Publ., Zoology **123**, 1–161.

Raymond, M.; Rousset, F. (1995): GENEPOP (version 1.2): population genetics software for exact test ecumenism. J. Hered. **86**, 248–249.

Reig, O. A. (1970): Ecological notes on the fossorial Octodont rodent *Spalacopus cyanus* (Molina). J. Mammalogy **51**, 592–601.

Reig, O. A.; Busch, C.; Ortells, M. O.; Contreras, J. R. (1990): An overview of evolution, systematics, population biology, cytogenetics, molecular biology and speciation in *Ctenomys*. In: Evolution of subterranean mammals at the organismal and molecular levels. Ed. by E. Nevo and O. A. Reig. New York: Wiley-Liss. Pp. 71–96.

Rogers, J. S. (1972): Measures of genetic similarity and genetic distance. Studies in Genetics VII. Univ. Texas Publ. **7213**, 145–153.

Sage, R. D.; Contreras, J. R.; Roig, V. G.; Patton, J. L. (1986): Genetic variation in South American burrowing rodents of the genus *Ctenomys* (Rodentia: Ctenomyidae). Z. Säugetierkunde **51**, 158–172.

Selander, R. K.; Smith, M. H.; Yang, S. Y.; Johnson, W. E.; Gentry, J. B. (1971): Biochemical polymorphism and systematics in the genus *Peromyscus*. I. Variation in the old-field mouse (*Peromyscus polionotus*). Studies in Genetics VII. Univ. Texas Publ. **7103**, 49–90.

Slatkin, M. (1985): Rare alleles as indicators of gene flow. Evolution **39**, 53–65.

– (1987): Gene flow and the geographic structure of natural populations. Science **236**, 787–792.

– (1993): Isolation by distance in equilibrium and non-equilibrium populations. Evolution **47**, 264–279.

Sprechmann, P. (1978): The paleoecology and paleogeography of the Uruguayan coastal area during the neogene and quaternary. Zitteliana **4**, 3–72.

STATISTICA. (1995): Statistica Release 5 for Windows. Statsoft, Inc. Tulsa, Oklahoma.

Swofford, D. L.; Selander, R. B. (1981): BIOSYS-1: a FORTRAN program for the comprehensive analysis of electrophoretic data in population genetics and systematics. J. Hered. **72**, 281–283.

Templeton, A. R. (1981): Mechanisms of speciation-a population genetic approach. Ann. Rev. Ecol. Syst. **12**, 23–48.

Weir, B. S.; Cockerham, C. C. (1984): Estimating F-statistics for the analysis of population structure. Evolution **38**, 1358–1370.

White, M. J. D. (1978): Modes of speciation. San Fransico: W. H. Freeman and Comp.

Wright, S. (1931): Evolution in mendelian populations. Genetics **16**, 97–159.

– (1969): Evolution and the genetics of populations. The theory of gene frequencies. Chicago: Univ. Chicago Press.

Authors' addresses: Guillermo D'Elía (current), University of Michigan, Museum of Zoology, Mammal Division, 1109 Geddes, Ann Arbor, MI 48109-1079, USA; Enrique P. Lessa, Laboratorio de Evolución, Facultad de Ciencias, Casilla 12106, Montevideo 11300, Uruguay; Joseph A. Cook, University of Alaska Museum, 907 Yukon Drive, Fairbanks, AK 99775-6960, USA.